PB90-100601

Inexpensive and Rapid Risk Assessment
Current Status and Future Directions

(U.S.) Environmental Protection Agency
Washington, DC

1990

U.S. Department of Commerce
National Technical Information Service
NTIS

1. REPORT NO. [illegible]	2. [illegible]
4. TITLE AND SUBTITLE: Detecting and Quantifying Aneuploidy: Current Status and Future Directions (Journal Version)	5. REPORT DATE: [illegible] 1991
7. AUTHOR(S): Ellen L. Mahlum and Gunnar Rosenkranz	8. PERFORMING ORGANIZATION REPT. NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Reproductive Effects Assessment Group, Office of Health & Environmental Assessment (RD-689), Office of Research & Development, U.S. Environmental Protection Agency, Washington, DC	10. PROJECT/TASK/WORK UNIT NO.
	11. CONTRACT/GRANT NO. [illegible]
12. SPONSORING AGENCY NAME AND ADDRESS: Office of Health & Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC 20460	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE: EPA/600/21

15. SUPPLEMENTARY NOTES: Environ. Mutagenesis, Teratogenesis, Carcinogenesis, Vol 1991
[illegible] Reproductive Effects Assessment Group, U.S. EPA; [illegible] Oak Ridge National Laboratory, Oak Ridge, TN; [illegible] Medical Department, Brookhaven National Laboratory, Upton, NY

16. ABSTRACT

The U.S. Environmental Protection Agency sponsored a workshop to discuss (1) the contribution of aneuploidy to human disease and disability, (2) the development of tests for detecting chemicals that induce aneuploidy and the relevance of these tests to human risk, and (3) the current understanding of mechanisms by which aneuploidy arises. This summary is based on the presentations given at the workshop. It is hoped that this summary will stimulate thinking in this vitally important area of risk assessment and contribute to the establishment of priorities for basic research, development of new test methods, and validation of existing test approaches. Such research is needed to enhance the scientific basis of risk assessment for aneuploidy-producing chemicals.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

AVAILABILITY BY NATIONAL TECHNICAL INFORMATION SERVICE U.S. DEPARTMENT OF COMMERCE SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to the public	Unclassified	21
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

[illegible]

Environmental Mutagenesis 7:669-682 (1985)

Aneuploidy and Health Risk Assessment: Current Status and Future Directions

Vicki L. Dellarco, Kathleen H. Mavournin, and Raymond R. Tice

Reproductive Effects Assessment Group, U.S. Environmental Protection Agency, Washington, DC (V.L.D.); Environmental Mutagen, Carcinogen, and Teratogen Information Program, Oak Ridge National Laboratory, Oak Ridge, Tennessee (K.H.M.); *and Medical Department, Brookhaven National Laboratory, Upton, New York (R.R.T.)*

The U.S. Environmental Protection Agency (EPA) recently sponsored a workshop[†] to discuss (1) the contribution of aneuploidy to human disease and disability, (2) the development of tests for detecting chemicals that induce aneuploidy and the relevance of these tests to human risk, and (3) the current understanding of mechanisms by which aneuploidy arises. This summary is based on the presentations given at the workshop. It is hoped that this summary will stimulate thinking in this vitally important area of risk assessment and contribute to the establishment of priorities for basic research, development of new test methods, and validation of existing test approaches. Such research is needed to enhance the scientific basis of risk assessment for aneuploidy-producing chemicals.

Key words: aneuploidy, review, genetic toxicology

[illegible]

[*]Operated by Martin Marietta Energy Systems, Inc., under contract No. DE-AC05-84OR21400 with the Department of Energy.

[†]The workshop, entitled "Approaches for Assessing the Risk of Aneuploidy Induction by Chemicals," was sponsored by the Reproductive Effects Assessment Group of the EPA and held at the National Academy of Sciences, Washington DC, October 3-4, 1983. The organizing committee was A. Hollaender (Council for Research Planning in Biological Sciences (CRPBS), Washington DC), F.E. Vrytek (EPA), R.R. Tice (Brookhaven National Laboratory), V. Dellarco (EPA), and C. Wilson (CRPBS). The speakers were B.R. Brinkley (Baylor College of Medicine, TX), W.E. Cande (University of California, Berkeley), R.F. Ozoli (Oak Ridge National Laboratory, TN), M. Hatch (Columbia University, NY), E.B. Hook (New York State Department of Health), R. Irons (Chemical Industry Institute of Toxicology, NC), E. Jackson (EPA), K.S. Lavappa (EPA), J.C. Liang (University of Texas), R.L. Margolin (Hutchinson Cancer Center, WA), K. Mavournin (Oak Ridge National Laboratory, TN), J.M. Parry (University of Swansea, U.K.), L.B. Russell (Oak Ridge National Laboratory, TN), S. Zimmering (Brown University, RI) and F.K. Zimmermann (Genetik, Technische Hochschule, West Germany). Other participants included J. Allen (EPA), R. Hill (EPA), J. Ivr (DuPont Co.), V. Meyer (Food and Drug Administration), S. Sandhu (EPA), and M. Shelby (National Toxicology Program).

Received January 11, 1985; revised and accepted February 13, 1985.

Address reprint requests to Dr. Vicki L. Dellarco, Reproductive Effects Assessment Group (RD-689), U.S. Environmental Protection Agency, 401 M Street SW, Washington, DC 20460.

The views expressed in this document are those of the authors and do not necessarily reflect the views and policies of the U.S. Environmental Protection Agency.

© 1985 Alan R. Liss, Inc.

NTIS is authorized to reproduce and sell this report. Permission for further reproduction must be obtained from the copyright owner.

[illegible]

Aneuploidy is the condition whereby cells or organisms suffer the loss or gain of one or more whole chromosomes. A principal event leading to aneuploidy is nondisjunction: the failure of homologous chromosomes to segregate normally in meiosis or in mitosis. However, the mechanisms by which chemicals can interfere with normal patterns of chromosome/chromatid segregation are not understood well [Brinkley and Chmelar, 1981; Hansmann, 1981]. Components of the spindle apparatus and other factors required for spindle function must be considered important cellular targets for inducing aneuploidy either in meiosis or mitosis. Chromosome pairing and crossing-over are additional targets for the induction of aneuploidy in meiosis.

Although nondisjunction in *Drosophila* was discovered some 70 years ago [Bridges, 1916], it was another forty years before its involvement in human health was recognized. In 1959, Lejeune and coworkers reported that Down syndrome is attributable to the presence of an extra chromosome [1]. The relationship of aneuploidy to human fetal wastage and clinical disorders is now well documented [Hook, 1981]. Klinefelter syndrome, Turner syndrome, Patau syndrome, and Edward syndrome are examples of aneuploid disorders. Furthermore, approximately 30% of early fetal wastage and about 5–7% of infant deaths result from aneuploidy [Hook, 1981]. Although the consequences of meiotic nondisjunction are clear, the extent to which mitotic nondisjunction in the early embryo contributes to human disorders is uncertain and should be explored further. Also, there is increasing evidence that mitotic aneuploidy may be a factor in the etiology of somatic cell malignancy [Rondo et al, 1984; Trakhtenbrot et al, 1983; Cavenee et al, 1983]. Because aneuploid conditions affect adversely the quality of life, the possible induction of aneuploidy by chemical substances is an important aspect of the total assessment of potential genetic hazards to humans. Little research and development effort, however, has been directed toward risk analysis for induced aneuploidy.

A variety of nonhuman test systems are used to identify mutagens [U.S. Environmental Protection Agency, 1984; Waters and Auletta, 1981]. Traditional genetic toxicology tests evaluate the ability of chemicals to cause DNA damage, gene mutations, or chromosome breakage and rearrangement. Because of mechanistic differences, however, the induction of aneuploidy might not be correlated with the induction of other types of genetic alterations. Therefore, chemicals that produce aneuploidy might not be detected by most mutagenicity assays. Efforts are being made to develop and validate short-term tests to detect aneuploidy in a variety of organisms.

Results from experimental systems are useful for making qualitative judgments of the potential of an agent to induce mutagenic effects in humans. Test method systems relevant to humans can be used also to make quantitative population risk estimates. Mammalian in vivo tests for heritable effects are most desirable for quantitative estimates of transmissible effects in humans. The numerical-sex chromosome loss test in mice [Russell, 1979] is one potentially useful test system for quantitatively assessing risks of induced meiotic aneuploidy in germ cells.

Quantification of risk is necessary to meet the regulatory responsibilities of the federal government [Voytek, 1982]. Risk-assessment approaches and guidelines proposed for mutagenicity [U.S. Environmental Protection Agency, 1984; National

Academy of Sciences, 1982] cover gene mutations and chromosome breakage but not aneuploidy. Guidelines for aneuploidy are not yet feasible because of a lack of well-validated test methods and limited understanding of underlying mechanisms. A U.S. EPA workshop was convened to explore means to address these deficiencies.

POTENTIAL TARGETS FOR THE CHEMICAL INDUCTION OF ANEUPLOIDY DURING MITOSIS

An understanding of the mechanisms underlying chromosome disjunction and nondisjunction is essential to develop reliable model systems for the prediction of human risk due to exposure to aneuploidy inducing chemicals. The mitotic apparatus is a key component responsible for chromosome segregation and thus is a major target for chemical interference.

Major functional components of the mitotic apparatus are (1) spindle poles, (2) centromeric or kinetochore (KT) regions of the chromosomes, and (3) microtubules (MTs). The MTs, composed of tubulin subunits, are the major fibrous element in the spindle, observable from prophase through anaphase. Spindle MTs are of two classes, those that attach to chromosomes at the kinetochore (KT-MTs) and those that radiate out from the spindle poles and form the body of the spindle and asters (interpolar-MTs) [McIntosh, 1984]. There is substantial redundancy in the spindle body. Human fibroblasts (WI-38), for instance, have a total of about 1,600 MTs, including 800 interpolar-MTs and 800 KT-MTs (10 MTs per chromosome) [McIntosh and Landis, 1971]. It is reasonable, therefore, to expect that the spindle could function normally with fewer MTs, and this has been shown to be the case. For example, lowering the temperature during cell growth reduces the number of MTs. Under these conditions, chromosome movement is slower, but otherwise proceeds normally [Inoué, 1964]. Consequently, chemicals could reduce the number of MTs to some extent without affecting spindle function until some threshold level is reached.

Other components that may be important targets for chemical interference in spindle function include the sheath of membrane surrounding the spindle, cAMP-dependent kinases, and calmodulin (involved in calcium regulation and localized at spindle poles and kinetochores). Thus, a variety of potential cellular targets exists for chemical interference with chromosome segregation.

Cellular and chromosomal events of mitosis are summarized in Table 1. Several of these events are points at which failure could lead to the maldistribution of chromosomes. Centriole replication and separation occurring in the S to G_2 phases of the cell cycle establishes the bilateral symmetry of the spindle. Early in prophase, microtubules assemble to form the body of the spindle. The early destruction of this bipolarity can result in monopolar or multipolar spindles [Inoué, 1981]. Kinetochore replication during S phase and kinetochore activation for association with KT-MTs during prometaphase are other events that direct chromosome movement and ensure that chromosomes segregate with fidelity. Experiments with laser microbeams have shown that destruction of kinetochores interferes with chromosome movement [Brenner et al, 1980; McNeil and Berns, 1981]. Mitomycin C causes kinetochores to separate from chromosomes [Brinkley and Shaw, 1970]; the detached chromosome could be left behind and ultimately lost when the kinetochore moves to the pole. Human antibody to the kinetochore region has been used as a probe to study the

[illegible]

Stage	Events
G_1	[illegible]
S	[illegible]
G_2	[illegible]
Prophase	[illegible]
Pro-metaphase	[illegible]
Metaphase	[illegible]
Anaphase A	[illegible]
Anaphase B	[illegible]
Telophase	[illegible]
Cytokinesis	[illegible]

[illegible]

biology of the kinetochore [Brenner et al, 1981], but much of the structure and regulation of these microtubule organizing centers remains unknown.

The spindle appears to be inactive at metaphase. A cellular signal, probably involving calcium levels interacting with the centromeric regions of the chromosomes, triggers separation of sister chromatids [Izant, 1983]. Chemicals could either interfere with the reception of this signal or the timing of the reception and thus cause malsegregation. It has been found that premature centromere separation is related to aneuploidy [Vig, 1984].

Anaphase is characterized by two events: the movement of chromosomes to the poles, which involves the shortening of KT-MTs (referred to as anaphase A), and spindle elongation, which involves the growth and rearrangement of interpolar-MTs (referred to as anaphase B) [Ris, 1949; Inoué, 1981; Brinkley and Cartwright, 1971; McIntosh, 1984]. Anaphase A involves pulling the chromosomes to the poles, whereas anaphase B involves pushing the poles apart; these are physiologically distinct pro-

centers in that anaphase A is ATP-independent whereas anaphase B is ATP-dependent [Cande, 1982]. *The two can be distinguished also by selective drug treatment.* Taxol, *which stabilizes MTs, inhibits chromosome-to-pole movement but does not interfere with spindle elongation* [Cande et al, 1981]. *Chloral hydrate blocks spindle elongation but not chromosome-to-pole movement* [Rieder, 1982]. *Therefore, agents that either destabilize or stabilize MTs could cause aneuploidy.*

POTENTIAL TARGETS FOR THE CHEMICAL INDUCTION OF ANEUPLOIDY DURING MEIOSIS

Events that could lead to aneuploidy in the first division of meiosis are more numerous than those of mitosis. There are three interdependent processes involved in proper chromosome distribution in the first division of meiosis. These include (1) homologous pairing (synapsis), (2) recombination (crossing-over), and (3) segregation. The first two do not involve spindle effects, whereas malsegregation at either meiosis I or meiosis II may involve spindle or kinetochore malfunction. In both Drosophila and humans, spontaneous nondisjunction during meiosis I occurs more often than in meiosis II [Bridges, 1916; Sanger et al, 1977; Hansmann, 1979; Merriam and Frost, 1964], *implying that pairing and recombination processes are sensitive to malfunction.*

The synaptonemal complex (SC), although its function is not fully understood, is thought to hold homologues in close proximity to facilitate recombination [Moses, 1968]. *If so, the SC may be a target for chemical interference. It is widely believed that the appearance of an SC and the alignment of homologues occur at the beginning of the zygotene stage of meiotic prophase* [Moses, 1968; Moses and Poorman, 1984; Moses, 1985]. *However, it has been reported from evidence in Drosophila, in the mouse, and in wheat that synapsis begins during premeiotic interphase* [Grell and Generoso, 1982; Grell et al, 1980; McQuade and Pickles, 1980]. *The timing of synapsis may define a period of increased sensitivity for chemical effects on the SC.*

Recombination between homologous chromosomes appears to be important for normal segregation. In *Drosophila melanogaster* oocytes, when homologous chromosomes pair but fail to undergo crossing-over, they enter separately into a so-called "distributive pool" of non-cross-over chromosomes [Grell, 1962]. If nonhomologous chromosomes then pair (so-called "distributive pairing"), abnormal patterns of chromosome segregation can result [Grell, 1962; Grell, 1976]. Chromosome rearrangements (eg, inversions, translocations) increase the incidence of distributive pairing and, thus, the frequency of aneuploid products. The distributive pairing model for *Drosophila* has been used to explain in humans [Grell, 1971; Grell and Valencia, 1964] the occurrence in a kindred of aneuploidy associated with translocations of unrelated chromosomes [Dutrillaux and Lejeune, 1970; Balkan and Martin, 1983; Moorehead et al, 1961].

TEST DEVELOPMENT

Several methods have been devised to detect mitotic and meiotic aneuploidy in experimental organisms (Table II). However, aneuploidy tests have not yet been used enough to meet commonly accepted standards of test validation: (1) reproducibility of results within a given laboratory in tests with a large number of compounds; (2)

TABLE 6. Approaches to Detect Aneuploidy

Test systems	Cell type	Aneuploid type[a]	Method
Fungi			
Aspergillus	Mitotic	Loss/gain	Genetic
Neurospora	Mitotic	Gain	Genetic
Saccharomyces	Mitotic/meiotic	Loss/gain	Genetic
Schizosaccharomyces	Mitotic	Gain	Genetic
Higher plants[b]			
Allium	Mitotic	Loss/gain	Cytological
Vicia	Mitotic	Loss/gain	Cytological
Hordeum	Mitotic/meiotic	Loss/gain	Cytological
Tradescantia	Mitotic/meiotic	Loss/gain	Cytological
Insects			
Drosophila	Meiotic	Loss/gain	Genetic
Mammalian cells in culture (established primary)	Mitotic	Loss/gain	Cytological
Whole mammal somatic cells (rodent or human)	Mitotic	Loss/gain	Cytological
Whole mammal female germ cells (rodents)	Meiotic	Loss/gain	Cytological
Whole mammal male germ cells (rodents)	Meiotic	Loss/gain	Cytological, genetic

[a]Chromosome gain is considered to be the critical diagnostic aneuploid type, because loss may be due to artifacts or clastogenic effects.
[b]Although not discussed in the workshop, plant systems can play an important role in the detection of aneuploidy-inducing agents.

agreement between different laboratories testing a common set of chemicals with the same protocol; (3) an acceptable level of concordance with other test systems involving different organisms; and (4) confirmation of the ability to detect the end point in question. Accordingly, descriptions of the tests provided below are limited to major perceived attributes, anticipated difficulties, and, where available, corrective measures to handle the latter.

Genetic tests in fungi are capable of detecting aneuploidy in mitotic and meiotic cells [reviewed by Griffiths, 1982; Zimmermann, 1983]. The fungal systems permit aneuploidy to be distinguished from other genetic alterations by simple genetic tests. It is not known whether biotransformation is a prerequisite for the induction of aneuploidy by some chemicals; however, the assays can be conducted both in the presence and in the absence of exogenous metabolic activation.

In insects, methods for detecting aneuploidy have involved predominantly *Drosophila melanogaster*, a complex organism with germ-cell stages similar to those of mammals. The principal approach utilizes genetically marked sex chromosomes and measures for sex chromosome nondisjunction. For technical reasons, methods to monitor nondisjunction of autosomes have not been used widely in testing. However, frequencies of x-ray-induced and spontaneous sex chromosome and autosome nondisjunction are similar despite wide disparities in chromosome size [Morgan et al, 1925; Grell et al, 1966]. Thus, there is no compelling reason to regard sex chromosome nondisjunction frequencies as unrepresentative of the genome as a whole. Females may be more appropriate as a test organism for nondisjunction since meiosis I in the

male *Drosophila melanogaster* is aberrant; ie, pairing takes place only at restricted heterochromatic conjunctive sites [Cooper, 1964] and recombination normally does not occur. Thus, the female *Drosophila* would better approximate the meiotic process of humans than the male *Drosophila*. Nonetheless, there is no experimental evidence to justify exclusion of the male as a test organism for aneuploidy detection [Fouremen, 1985]. Aneuploidy screens under development in *Drosophila* make use of special selective schemes that permit far larger sample sizes to be obtained per unit of time and accordingly provide a firmer data base for judgments regarding the aneuploidy-inducing ability of tested chemicals. Valencia et al [1984], Zimmering et al [1985] and Fouremen [1985] have recently reviewed some of the various test approaches used in *Drosophila*.

Another insect system using grasshopper embryos has been proposed for detecting effects of volatile chemicals on mitotic disjunction [Liang et al, 1983]. This test system uses mitotic arrest as an indication of the potential of chemicals to induce aneuploidy.

Detection of aneuploidy in mammalian cell culture systems is based primarily on mitotic metaphase chromosome counts. Although established cell lines (particularly Chinese hamster lines, eg, CHO and V79) have been used, a high and variable background frequency of aneuploidy may reduce the sensitivity of such material. The use of primary cell cultures would eliminate this problem. Although there appears to be agreement that scoring for hyperploidy is a reliable measure of aneuploidy, considerable doubt exists regarding the significance of hypoploid cells since technical artifacts during slide preparation may result in hyploid cells. Danford [1984] has developed a procedure to minimize the occurrence of chromosome loss during hypotonic treatment. Replication kinetics must be considered also in the development of a reliable test approach for cells in culture. Cells must be examined for aneuploidy after sufficient time has elapsed after exposure for one intervening round of cell division, yet before death of many aneuploid progeny has occurred. Assays can be carried out with and without metabolic activation.

Other test approaches have been developed in mammalian cells using end points other than direct chromosome counts. Parry et al [1982] have adapted a method for detecting malsegregation by differently staining mammalian cells in vitro for chromosomes and spindle fibers. Aneuploid cells have been identified by measuring DNA content using flow cytometry [Barlogie et al, 1983; Rabinovitch et al, 1982]. However, flow cytometry may not be able to distinguish aneuploidy from clastogenic events.

As with mammalian cells in culture, the most common approach used to detect aneuploidy in somatic cells of whole mammals has been by direct cytological analysis of the chromosome complement of metaphase cells. Such studies have been conducted primarily in rodents using bone marrow cells. Aneuploidy in somatic cells has been inferred also from the induction of micronuclei in bone marrow cells [Heddle et al, 1983]. Because micronucleus analysis cannot discriminate between aneuploidy and chromosome breakage, concomitant proof that chromosomal aberrations are not produced by the same chemical treatment is required.

Various in vivo analyses have been used for detecting aneuploidy in mammalian germ cells. These approaches primarily use the mouse and involve chromosomal analysis at meiotic metaphase I or metaphase II; fluorochrome analysis of spermatids; or chromosome analysis of zygotes, morula, blastocysts, midgestational embryos,

and newborns [Russell, 1979]. Based on known developmental effects of different trisomies, gross observations of day 12–19 mouse fetuses followed by cytological examination of the abnormals may be useful as an aneuploidy screen. The mouse numerical sex chromosome anomaly test using X-linked markers [Russell, 1979] and the use of mouse stocks bearing Robertsonian translocations [Cattanach et al, 1984] may be useful genetic approaches for detecting aneuploidy induction.

A tier testing approach for detecting aneuploidy-inducing chemicals, using Chinese hamster DON fibroblasts in vitro, mouse bone marrow, and germ cells in vivo, was proposed by Dr. J.C. Liang during the workshop described in this paper. Data from somatic cell studies using colcemid, vinblastine, and diazepam were presented to demonstrate the detection of aneuploidy-inducing agents by anaphase to metaphase ratios, mitotic indices, and chromosome counts [Hsu et al, 1983a,b; Liang and Satya-Prakash, 1985]. Other test strategies using fungi and *Drosophila* in addition to mammalian systems could be proposed.

Environmental Health Perspectives [Vol 31, August 1979] and the Aneuploidy Data Review Committee* reports [Mutation Research, submitted] contain additional descriptions of methods to detect the chemical induction of aneuploidy.

HUMAN MONITORING

Populations can be monitored for germ-cell aneuploidy by cytogenetic analysis of cells from induced or spontaneous abortus material, umbilical cord blood or amniotic fluids, or by phenotypic survey of newborns, eg, Down syndrome [Hook, 1981, 1983]. Sensitivity and population size requirements limit the feasibility of such approaches to large population surveys.

In a retrospective study conducted by a Columbia University group, a correlation has been claimed between parental exposure and the frequency and relative proportions of specific karyotypic anomalies in spontaneous abortions. It was suggested that caffeine intake [Kline et al, 1980], use of spermicides [Strobino et al, 1980], and parental occupations as domestic workers (for females) or university professors (for males) [Hatch et al, 1984] were associated with an increased frequency of aneuploid abortuses. The relative power of this approach, using all spontaneous abortions up to 28 weeks of gestation, is limited by the small sample sizes generally available. Ascertainment of early gestational age abortuses only would increase the sensitivity. Surveys of this kind conducted prospectively in exposed populations and may usefully provide a basis for direct assessment of human risk.

COMPARISON OF ANEUPLOIDY WITH OTHER TYPES OF GENETIC ALTERATIONS

Data on the induction of aneuploidy were compared with data on the induction of gene mutations and cytogenetic damage for a selected list of compounds to

*A committee sponsored by the U.S. EPA to critique the current state-of-methodology for aneuploidy testing. This committee is directed by the Reproductive Effects Assessment Group (Dr. V. L. Dellarco, U.S. EPA, RD-689, 401 M Street, S.W., Washington, D.C. 20460) and Health Effects Research Laboratory (Dr. M.D. Waters, U.S. EPA, MD-68, Research Triangle Park, NC 27711).

determine if there are chemicals that cause aneuploidy as their predominant effect (Table III). Cell transformation data were included to ascertain whether any evidence exists presently for the hypothesis that one class of carcinogens acts by causing numerical chromosome abnormalities rather than by causing gene mutations. All data shown in Table III were obtained from the data base on genetic effects of chemicals compiled by the Gene-Tox Program [Waters and Auletta, 1981]. Chemicals were selected for which a positive, or "possibly positive," assessment of aneuploidy induction had been made by at least one Gene-Tox Work Group. This restriction limited the data base in Table III to aneuploidy bioassays using *Drosophila, Aspergillus, Neurospora, or Saccharomyces;* mammalian systems for detecting aneuploidy were not reviewed by the Gene-Tox Program. Moreover, all Gene-Tox assessments are based on results published before 1980, the literature available at the time the work groups began their reviews.

It is evident from an examination of Table III that some chemicals are active in systems with different genetic end points, notably mitomycin C, azathioprine, and several alkylating agents, eg, N-methyl-N'-nitro-N-nitrosoguanidine, ethyl methylsulfonate, ethylene dichloride, and the alkylnitrosoureas. These compounds clearly interact with DNA and are detected in standard mutagenicity tests. Gene-Tox reported no cell transformation assessments for mitomycin C or azathioprine, but both are tumorigenic, [mitomycin C: IARC Monographs 10:171, 1976; azathioprine: IARC Monographs 26:47, 1981].

It is apparent immediately that the data base is still very incomplete and cannot support adequately the intended analysis. Several known or suspected mitotic poisons (colchicine and colcemid, p-fluorophenylalanine, griseofulvin, and organic mercurials) induce aneuploidy but have received little or no testing for other genetic effects. Other compounds give positive or suggestive results in aneuploidy bioassays and conflicting or weak responses in mutagenicity tests: actinomycin D, caffeine, DDT, 5-fluorodeoxyuridine, and LSD. Based on the results in Table III, no compound can be identified unequivocally as positive for both aneuploidy induction and cell transformation and as weakly mutagenic or nonmutagenic. Drawing on more recent publications does little to reduce these inadequacies, but indicates several new candidates for aneuploid-inducing carcinogens. Trifluralin induces nondisjunction in *Drosophila* [Foureman, 1981] and tumors in female mice [NCI-CG-TR-34, 1984]; cadmium chloride causes aneuploid oocytes in mice [Watanabe and Endo, 1982] and cell transformation (Table III); neither compound was evaluated as mutagenic by Gene-Tox (Table III). Aneuploidy induced by benomyl has been observed in several systems: human lymphocytes in vitro [Tenchini et al, 1983], *Saccharomyces* [Sora et al, 1982; Alli and Parry, 1983], and *Aspergillus* [Gualandi and Bellincampi, 1981]. However, both benomyl and its metabolite, methyl 2-benzimidazole carbamate (MBC), are reportedly negative in nondisjunction and sex-chromosome-loss tests in *Drosophila* [Lamb and Lilly, 1980]. Data on mutagenicity and other genetic effects are scanty and inconclusive, but benomyl and MBC cause tumors in rats [U.S. Environmental Protection Agency, 1982].

For diethystilbestrol (DES), the situation is more ambiguous. The parent compound is negative for aneuploid induction in *Neurospora* [Brockman et al, 1984] but has been reported positive in cultured mammalian cells [Kinsella, 1982; Parry et al, 1982; Tsutsui et al, 1983] and in *Saccharomyces* [Parry et al, 1982]. The dipropionate ester is reported to be positive in yeast (Table III) and in cultured cells [Kinsella,

TABLE III. Comparison of the Induction of Aneuploidy with Other Genetic End Points: The Gene-Tox Data Base[a]

Chemical and CAS No.	Aneuploidy					Assays[a] gene mutations						Chromosome damage	Cell transformation
	As	Ne	Sa	DL	DG	Ba	Pl	Fu	Dr	MC	WM		
Acetylsulfanilamide 144-80-9		+	+										
Actinomycin D 50-76-0				+	+		+	+?	I	–		+	+
2-Amino-1,3,4-thiadiazole 4005-51-0				+	+							I	
Amphetamine sulfate 60-13-9				+?	+?				I				
Amphotericin B 1397-89-3	+												
Atrazine 1912-24-9		–		+	I		+	+	I			P	+
Azathioprine 446-86-6				+	+	+		+				+	
Benomyl 17804-35-2	+					I						P	
Betanal 13684-63-4	+												
BHT 128-37-0				+		–			–		–	P	
Cadmium chloride 10108-64-2				+		I						–	+
Caffeine 58-08-2		+	+	+	+	–	+		+	–	–	+	I
Chloral hydrate 302-17-0	+	–				+						I	
Colcemid 477-30-5				+	+							ND	
Colchicine 64-86-8		–		+	+		+					ND	

TABLE III. Comparison of the Induction of Aneuploidy with Other Genetic End Points: The Gene-Tox Data Base[a] (continued)

Chemical and CAS No.	Aneuploidy					Assays[a] gene mutations						Chromosome damage	Cell transformation
	As	Ne	Sa	DL	DG	Ba	Pl	Fu	Dr	MC	WM		
Cyanazine 21725-46-2		−		−	I		+		I			P	
1,4-Dichloro-2,5-dimethoxy-benzene 2675-77-6	+												
1,3-Dihydroxyurea 686-68-0				−	+?				I				
Diethylstilbestrol dipropionate 130-80-3			+										
Dimethylsulfate 77-78-1				+		+	+	+	+	+		+	
3-(3,3-Dimethyl-1-triazenyl) pyridine 1-oxide 21600-42-0				+		+			+			+	
3,3-Dimethyl-2-(2,4,6-trichloro-phenyl)-triazene 50355-74-3				+?				+	+			M	
DMSO 67-68-5	+	−		−		−			−			−?	−
p,p'-DDT 50-29-3				I	+?	−			−	+		+	
Econazole 27220-47-9	+												
Ethylene dichloride 107-06-2				+	+	+			+				+
Ethylmethylsulfonate 62-50-0		−	+			+	+	+	+	+	+	+	+
Ethylnitrosourethane 614-95-9				+	+?	+	+	+	+		+	+	

continued

TABLE III. Comparison of the Induction of Aneuploidy with Other Genetic End Points: The Gene-Tox Data Base[a] (continued)

Chemical and CAS No.	Aneuploidy					Assays[b] gene mutations						Chromosome damage	Cell transformation
	As	Nc	Sa	DL	DG	Ba	Pl	Fu	Dr	MC	WM		
Ethyl thiophanate 23564-06-0	+												
N-Ethyl-N-nitrosourea 759-73-9				+		+	+	+	+	+	+	+	+
FUdR 50-91-9	I	–		+	+	–	+		I	–		P	+
p-Fluorophenylalanine 60-17-3	+	+	+					+					
Griseofluvin 126-07-8	+					I						P	
Heliotrine 303-33-3				+	–?			+	+			P	
Hydroxylamine 7803-49-8				–	+?			+	I			P	+
Hydroxymethyl mercury 1184-57-2				+	+							P	
Isopropyl methane-sulfonate 926-06-7				+	–		+	+	+	+	I	+	
LSD 50-37-3				+	+		–		+			I	
Lucanthone 548-57-2		–		+	–?	+				+			–
Merbromin 129-16-8			+										
Methotrexate 59-05-2		+				+	–			+		+	I
Methyl benzimidazole carbamate 10605-21-7	+										+?	P	

continued

TABLE III. Comparison of the Induction of Aneuploidy with Other Genetic End Points: The Gene-Tox Data Base[a] (continued)

Chemical and CAS No.	Aneuploidy					Assays[a] gene mutations						Chromosome damage	Cell transformation
	As	Ne	Sa	DL	DG	Ba	Pl	Fu	Dr	MC	WM		
Methyl thiophanate 23564-05-8	+											–	
Methoxyethylmercury chloride 123-88-6				+?	I								
4-Methoxy-1,3-phenylene-diamine sulfate 6219-67-6		+							+				
N-Methyl-N'-nitro-nitrosoguanidine 70-25-7		–			+	+	+	+	+	+	+?	+	+
Methyl-nitrourea 684-93-5			+	+	–	+	+	+	+	+	+	+	
Mitomycin C 50-07-7		–	+		–	I	+	+	+	+	+	+	
Phenylmercury acetate 62-38-4				+	+								
Procarbazine 366-70-1				+	–	+		+	+	+	+	+	+
Proflavin 92-62-6				+	+?	+				+		S	
Saccharin 81-07-2		–	+			+		+		I		+	–
Sodium fluoride 7681-49-4		–		+	–	I			I			P	
Sulfur mustard 505-60-2				+		+		+	+	+		+	
2,3,5,6-Tetrakis (1-aziridinyl)-p-benzoquinone 4239-06-9				+			+	+			I		

—continued

TABLE III. Comparison of the Induction of Aneuploidy with Other Genetic End Points: The Gene-Tox Data Base* (continued)

Chemical and CAS No.	Aneuploidy					Assays[a] gene mutation						Chromosome damage	Cell transformation
	As	Ne	Sa	DL	DG	Ba	Pl	Fu	Dr	MC	WM		
Thiabendazole 148-79-8	+							I					
Thimerfonate sodium 5964-24-9			+										
Thiophanine 27079-29-4	+												
Triethyllead chloride 1067-14-7				+	−								
Trifluralin 1582-09-8		+				−			−			P	
Trimethoprim 738-70-5		+											
Urethane 51-79-6				+	−	−		+	+			+	+

*The information regarding aneuploidy presented in this table is currently being reviewed by the U.S. EPA Aneuploidy Data Review Committee, and thus some results may change after re-evaluation.

[a] +, positive; −, negative; I, inconclusive; +?, probably positive; −?, probably negative; G, chromosome gain; L, chromosome loss; M, positive results in micronucleus test only; S, positive results for SCE only; P, positive results only in plant systems. Abbreviations: As = *Aspergillus*; Ne = *Neurospora*; Sa = *Saccharomyces*; DL = *Drosophila* loss; DG = *Drosophila* gain; Ba = bacteria; PL = plants; Fu = Fungi; Dr = *Drosophila*; MC = mammalian cells in culture; WM = whole mammals.

1982]. The diphosphate ester, however, has been reported positive [Chrisman and Hinkle, 1974] and negative in murine bone marrow [Ivett and Tice, 1981], positive in mouse embryos treated in utero [Chrisman, 1974], and negative in mouse oocytes treated in vivo [Becker and Schoneich, 1981]. Tests for mutagenicity are contradictory [cf, Gene-Tox reports], but DES is a human carcinogen [IARC Monographs 21:173, 1979].

Clearly the "state-of-the-literature" on aneuploidy bioassays lags behind what has been accomplished in recent years for other types of genetic tests. A review of published data on plant and mammalian systems and an update of the data base for fungal and *Drosophila* tests are essential before we can begin to address several critical questions. Is aneuploidy a genetic risk undetected by tests for gene mutation or chromosomal breakage? How reliably do results from mitotic sysems predict meiotic nondisjunction? Could results from lower eukaryotes be used to predict mammalian, including human, aneuploidy? Further testing of the compounds mentioned above is clearly needed. What we can learn most profitably from Table III is which of the blank spaces and question marks should be filled in first, by a combination of library work and laboratory research, to provide the core of a truly useful table. The Aneuploidy Data Review Committee, established since the workshop described in this paper, has undertaken the task of evaluating the current state-of-test-methodology; their reports will be available in the near future. Assessments made by this committee will be based in part on information unavailable at the time of the Gene-Tox review and may differ from the Gene-Tox assessments presented in Table III.

RISK ASSESSMENT

Health risk assessments are used by the U.S. EPA as a major component of decisions on the regulation of mutagens, carcinogens, or other toxic agents. Risk assessment can be viewed as a two-step process. The first step is a qualitative evaluation of toxicological data to determine the potential hazard of a chemical substance to humans. This evaluation is expressed in terms of weight-of-the-evidence, largely from results of short-term tests. Specific recommendations of tests for the identification of chemicals that may cause aneuploidy in mammals have not been made because aneuploidy tests have not yet been well validated.

The second step is an estimation of the magnitude of risk associated with human exposure to the agent. This quantitative step involves analysis of in vitro and in vivo data and extrapolation across species to human exposure situations. The types of extrapolations that must be made to estimate risk quantitatively may be generally the same for aneuploidy as for gene mutations. Assessments based on a whole mouse model usually require high- to low-dose extrapolations and species-to-species extrapolations. Assessments based on a human somatic cell model require somatic cell to germinal cell extrapolations.

Although the types of required extrapolations are the same for aneuploidy-producing agents and mutagens, the target molecules and mechanisms of induction are different. Except in special cases, DNA is the primary target molecule for mutagens. The target molecules and mechanisms of induction of aneuploidy are not well understood; interaction with any functional component of chromosome pairing, crossing-over, or segregation could be involved.

The variety of potential target sites increases the uncertainty in high- to low-dose extrapolations for aneuploidy induction. A dose-response curve with no threshold is often assumed in risk assessment for gene mutations [National Academy of Sciences, 1980; United Nations Scientific Committee on the Effects of Atomic Radiation, 1972]. In the case of aneuploidy, however, interaction with each target may have its own dose-response curve that may or may not include a threshold. When making high- to low-dose extrapolations for a particular chemical, knowledge of its target molecule(s), and information on the shape of the dose-response curve(s) will be needed.

Species-to-species extrapolations for the induction of aneuploidy may be complicated also by the number of target molecules. Such extrapolations for mutagens are based on the fact that the primary target, DNA, is essentially the same from species to species. It is not yet known whether the target molecules for aneuploidy-producing chemicals are sufficiently conserved to provide a similar basis for extrapolation. Because of the special features of meiosis, it is not yet known whether extrapolations from mitotic to meiotic cells (or from somatic to germ cells) are legitimate. Systematic comparison of tests will be essential in deciding the most appropriate models for prediction and estimation of human germ- and somatic-cell risks.

FUTURE RESEARCH DIRECTIONS

Research is needed to elucidate the various mechanisms involved in chemical effects on chromosome disjunction, differences between mitotic and meiotic cells, and differences between sexes and species. The possibility of a "no-effect threshold" requires critical consideration. The many potential sites for interference of cell division must be probed by a spectrum of agents. For example, agents that affect spindle formation by inhibiting or enhancing polymerization of tubulin, centriole separation, and kinetochore function, should be considered in test development. Agents with these selective actions need to be identified, if possible. A variety of test systems should be employed in the survey to determine the degree of universality.

Several administrative steps could be taken to facilitate test development and basic research. In particular, a committee is needed to integrate efforts of different parts of government and the private sector. A chemical repository for aneuploidy testing would be beneficial for comparative analysis of results collected from independent studies. Finally, meetings and symposia that bring together research and regulatory personnel for exchange of information and the coordination of plans should be encouraged.

There have been increased efforts in the areas of test development since the 1979 National Institute of Environmental Health Sciences (NIEHS) workshop on systems to detect the induction of aneuploidy by environmental agents [Zimmermann et al, 1979]. For example, efforts in this direction are being made in fungi and *Drosophila* under National Toxicology Program sponsorship. However, development of new mammalian test systems should be encouraged. Sensitive and cost-effective methods of assessing germinal and somatic risk in humans are needed. Prospective cytogenetic analysis of abortus material may be a useful germ-cell approach for monitoring human populations. Appropriate solvents, positive controls, standards for treatment times and spontaneous background frequencies, and rigorous statistical methods for analysis of the resultant data need to be established for all systems.

Metabolic activation of aneuploidy-producing agents is largely uninvestigated. The paucity of the existing data base limits comparative judgments of test validity. A systematic evaluation of a series of model chemicals in assays for both mitotic and meiotic aneuploidy in a range of organisms from fungi to mammals is needed to assess the reliability and sensitivity of aneuploidy tests.

ACKNOWLEDGMENTS

The U.S. EPA- and NIEHS-sponsored symposium entitled "Aneuploidy: Etiology and Mechanisms," held in Washington, DC, at the Carnegie Institution on March 25 to 29, 1985, and the U.S. EPA-sponsored "Aneuploidy Data Review Committee" developed directly from the workshop described in this paper. The symposium focused additional attention on the problem of human aneuploidy and possible mechanisms by which chemicals produce aneuploidy. The data review committee provided an in-depth analysis of the available test systems for chemically induced aneuploidy detection and of data generated using these systems. R.R. Tice is supported by the U.S. Department of Energy under contract No. DE-AC02-76CH00016, and K.H. Mavournin is supported by contract No. DW89930922-01-0. The workshop was sponsored by the Reproductive Effects Assessment Group, Office of Health and Environmental Assessment, Office of Research and Development, U.S. Environmental Protection Agency, under contract No. DW930022-01-1.

REFERENCES

Alli FN, Parry JM (1983): Studies on the induction of genetic damage in the yeast *Saccharomyces cerevisiae*. Environ Mutagen 5:248.

Balkan W, Martin RH (1983): Chromosome segregation into the spermatozoa of two men heterozygous for different reciprocal translocations. Hum Genet 63:345–348.

Barlogie B, Raber MN, Schumann J, Johnson TS, Drewinko B, Swartzendruber DE, Gohde W, Andreeff M and Freireich EJ (1983): Flow cytometry in clinical cancer research. Cancer Res 43:3982–3997.

Becker K, Schoneich J (1981): Effects of oestrogen on meiosis in female mice. Mutat Res 85:266.

Bond DJ, Chandley AC (1983): "Aneuploidy." Oxford Monographs on Medical Genetics. Oxford University Press, 198pp.

Brenner SL, Liaw LH, Berns MW (1980): Laser microirradiation of kinetochores in mitotic PTK_2 cells: Chromatid separation and micronucleus formation. Cell Bio Phys 2:139–152.

Brenner S, Pepper D, Berns MW, Tan E, Brinkley BR (1981): Kinetochore structure, duplication, and distribution in mammalian cells: Analysis by human autoantibodies from scleroderma patients. J Cell Biol 91:95–102.

Bridges C (1916): Nondisjunction as proof of the chromosome theory of heredity. Genetics 1:1–52; 107–163.

Brinkley BR, Cartwright J (1971): Ultrastructural analysis of mitotic spindle elongation in mammalian cells in vitro: Direct microtubule counts. J Cell Biol 50:416–531.

Brinkley BR, Shaw M (1970): Ulstrastructural aspects of chromosome damage. Genetic Concepts and Neoplasia Monograph. A collection of papers from the 23rd symposium on cancer research, University of Texas, M.D. Anderson Hospital and Tumor Institute. Baltimore: Williams and Wilkins, pp 313–345.

Brockman HE, de Serres FJ, Ong T-M, DeMarini DM, Katz AJ, Griffiths AJF, Stafford RS (1984): Mutations tests in *Neurospora crassa:* A report of the US Environmental Protection Agency Gene-Tox Program. Mutat Res 133:87–134.

Cande WZ, McDonald K, Meeusen RL (1981): A permeabilized cell model for studying cell division: A comparison of anaphase chromosome movement and cleavage furrow constriction in lysed PtK_1 cells. J Cell Biol 88:618–629.

Cande WZ (1982): Nucleotide requirements for anaphase chromosome movements in permeabilized mitotic cells: Anaphase B but not anaphase A requires ATP. Cell 28:15–22.

Cattanach BM, Papworth D, Kirk M (1984): Genetic tests for autosomal nondisjunction and chromosome loss in mice. Mutat Res 126:189–204.

Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, Murphree AL, Strong LC, While RL (1983): Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 305:779–984.

Chrisman CL (1974): Aneuploidy in mouse embryos induced by diethylstilbestrol diphosphate. Teratology 9:229–232.

Chrisman CL, Hinkle LL (1974): Induction of aneuploidy in mouse bone marrow cells with diethylstilbestrol-diphosphate. Can J Genet Cytol 16:831–835.

Cooper KW (1964): Meiotic conjunctive elements not involving chiasmata. Proc Natl Acad Sci USA 52:1248–1255.

Danford N (1984): Measurement of levels of aneuploidy in mammalian cells using a modified hypotonic treatment. Mutat Res 139:127–132.

Dutrillaux B, Lejeune J (1970): Etude de la decendance des individus porteurs d'une translocation t(DqDq). Ann Genet 13:11–19.

Foureman PA (1981): Identification of aneuploidy inducing chemicals in Drosophila. Environ Mutagen 3:319.

Foureman PA (1985): Detection of Aneuploidy in Drosophila. In de Serres FJ (ed): "Chemical Mutagens: Principles and Methods for Their Detection." New York: Plenum. Vol. 10 (in press).

Grell RF (1962): A new hypothesis on the nature and sequence of meiotic events in the female of *Drosophila melanogaster*. Proc Natl Acad Sci USA 48:165–172.

Grell RF (1971): Distributive pairing in Man? Ann Genet 14:165–171.

Grell RF (1976): Distributive pairing. In Ashburner E, Novitske E (eds): "Genetics of Biology of Drosophila." New York: Academic Press, Vol. 1a, pp 436–483.

Grell RF, Generoso EE (1982): A temporal study at the ultrastructural level of the developing pro-oocyte of *Drosophila melanogaster*. Chromosoma 87:49–75.

Grell RF, Munoz ER, Kirschenbaum WF (1966): Radiation-induced nondisjunction and loss of chromosomes in *Drosophila melanogaster* females. I. The effect of chromosome size. Mutat Res 3:494–502.

Grell RF, Oakberg EF, Generoso EE (1980): Synaptonemal complexes at pre-meiotic interphase in the mouse spermatocyte. Proc Natl Acad Sci USA 77:6720–6723.

Grell RF, Valencia JI (1964): Distributive pairing and aneuploidy in man. Science 145:66–67.

Griffiths AJF (1982): Short-term tests for chemicals that promote aneuploidy. In de Serres FJ, Hollaender A (eds): "Chemical Mutagens." New York: Plenum, Vol 7, pp 189–210.

Gualandi G, Bellincampi D (1981): Induced gene mutation and mitotic nondisjunction in *A. nidulans*. Toxicol Lett 9:389–394.

Hansmann I (1979): Clustering of chromosomal aneuploidy and tracing of nondisjunction in man. Environ Mutagen 31:23–25.

Hansmann I (1983): Factors and mechanisms involved in nondisjunction and X chromosome loss. Cytogenetics of the mammalian X chromosome, Part A Basic mechanisms of X chromosome behavior. New York: Alan R. Liss, pp 131–170.

Hatch M, Kline J, Stein Z, Warburton D, and Hutzler M (1984): Male risk factor for spontaneous abortions. Presented at Society for Epidemiological Research. Houston, Texas.

Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, Salamone MF (1983): The induction of micronuclei as a measure of genotoxicity: A report of the US Environmental Protection Agency Gene-Tox Program. Mutat Res 123:61–118.

Hook EB (1983): Perspectives in mutation epidemiology: Contribution of chromosome abnormalities to human morbidity and mortality and some comments upon surveillance of chromosome mutation rates. ICPEMC Working Paper 5/3. Mutat Res 114:389–423.

Hook EB (1981): Human teratogenic and mutagenic markers in monitoring about point sources of pollution. Environ Res 25:178–203.

Hsu TC, Liang JC, Shirley LR (1983a): Aneuploidy induction by mitotic arrestants: Effects of diazepam on diploid Chinese hamster cells. Mutat Res 122:201–209.

Hsu TC, Shirley LR, Takanari H (1983b): Cytogenetic assays for mitotic poisons: The diploid Chinese hamster cell system. Anticancer Research 3:155–160.

Inoué S (1981): Cell division and the mitotic spindle. J Cell Biol 91:131s–171s.
Inoué S (1964): Organization and function of the mitotic spindle. In Allen RD, Kamiyo N (eds): "Primitive Motile Systems in Cell Biology." New York: Academy Press. pp 549–598.
Ivett JL, Tice RR (1981): Diethylstilbestrol-diphosphate induces chromosomal aberrations but not sister chromatid exchanges in murine bone marrow cells in vivo. Environ Mutagen 3:445–452.
Izant JG (1983): The role of calcium ions during mitosis: Calcium participates in the anaphase trigger. Chromosoma 88:1–10.
Kinsella AR (1982): Elimination of metabolic cooperation and the induction of sister chromatid exchanges are not properties common to all promoting or cocarcinogenic agents. Carcinogenesis 3:499–503.
Kline J, Stein Z, Susser M, Warburton D (1980): Social economic differences in spontaneous abortions. Am Epidemiol 112:439–443.
Kondo K, Chilcote RR, Maurer HS, Rowley JD (1984): Chromosome abnormalities in tumor cells from patients with sporadic Wilms' tumor. Cancer Res 44:5376–5381.
Lamb MJ, Lilly LJ (1980): An investigation of some genetic toxicological effects of the fungicide benomyl. Toxicol 17:83–95.
Lejeune J, Gautier M, Turpin R (1959): Le mongolism, pre'mier e'xemple d'aberration autosomique humaine. CR Acad Sci (France) 248:1721–1722.
Liang JC, Hsu TC, Henry JE (1983): Cytogenetic assays for mitotic poisons: The grasshopper embryo system for volatile liquids. Mutat Res 113:467–479.
Liang JC, Satya-Prakash KL (1985): Induction of aneuploidy by mitotic arrestants in mouse bone marrow. Mutat Res 155:61–70.
McIntosh JR (1984): Mechanisms of mitosis. Trends in Biochemical Sciences. April:195–198.
McIntosh JR, Landis SC (1971): Distribution of spindle microtubules during mitosis in cultured human cells. J Cell Biol 49:468–497.
McNeil PA, Berns MW (1981): Chromosome behavior after laser microirradiation of single kinetochores in mitotic PTK_2 cells. J Cell Biol 88:543–553.
McQuade HA, Pickles DG (1980): Observations on synaptonemal complexes in premeiotic interphase of wheat. Am J Bot 67:1361–1373.
Merriam JR, Frost JN (1964): Exchange and nondisjunction of the X chromosome in female Drosophila melanogaster. Genetics 49:109–122.
Moorehead PS, Hellman WJ, Wenar C (1961): A familial chromosome translocation association with speech and mental retardation. Am J Human Genet 13:32.
Morgan TH, Bridges CB, Sturtevant AH (1925): The genetics of Drosophila. Bibliog Genet 2:1–262.
Moses MJ (1968): Chromosome structure-synaptonemal complex. Annu Rev Genet 2:363–412.
Moses MJ (1985): Composition and role of synaptonemal complex. In Evans CW, Dickenson HG (eds): "Controlling Events in Meiosis." 38th Symposium of Society for Experimental Biology, September 1983. Company of Biologist Limited, Cambridge, England (in press).
Moses MJ, Poorman PA (1984): Synapsis, synaptic adjustment and DNA synthesis in mouse oocytes. Chromosomes Today 8:90–103.
National Academy of Sciences (1980): The effects on populations of exposure to low levels of ionizing radiation. National Research Council, National Academy of Sciences, Washington, DC, pp 106–108.
National Academy of Sciences (1982): Identifying and estimating the genetic impact of chemical environmental mutagens. National Academy Press. Washington DC, 316pp.
Parry EM, Danford N, Parry JM (1982): Differential staining of chromosomes and spindle and its use as an assay for determining the effect of diethylstilboestrol on cultured mammalian cells. Mutat Res 105:243–252.
Rabinovitch PS, Martin GM, Hoehn H (1982): Interphase flow-cytogenetics: Correlation of DNA fluorescence to aneuploidy in human fibroblast cultures. Hum Genet 61:246–249.
Ris H (1949): The anaphase movement of chromosomes in the spermatocytes of the grasshopper. Biol Bull 96:90–106.
Russell LB (1979): Meiotic nondisjunction in the mouse: Methodology for genetic testing and comparison with other methods. Environ Health Perspect 31:113–129.
Sanger R, Tippett P, Gavin J, Teesdale P, Daniels GL (1977): Xg groups and sex chromosome abnormalities in people of northern European ancestry: An addendum. J Med Genet 14:210–211.
Sora S, Lucchini G, Magni GE (1982): Meiotic diploid progeny and meiotic nondisjunction in *Saccharomyces cerevisiae*. Genetics 101:17–32.

Strobino B, Kline J, Stein Z, Susser M (1980): Exposure to contraceptive creams, jellies, douches and their effects on zygotes. Am J Epidemiol 112:434–438.

Tenchini ML, Mottura A, Velicogna M, Pessina M, Rainaldi G, De Carli L (1983): Double Y as an indicator in a test of mitotic nondisjunction in cultured lymphocytes. Mutat Res 121:139–146.

Tsutsui T, Maizumi H, McLachlan JA, Barrett JC (1983): Aneuploid induction and cell transformation by diethylstilbestrol: A possible chromosomal mechanism in carcinogenesis. Cancer Res 43:3814–3821.

United Nations Scientific Committee on the Effects of Atomic Radiation (1972): Ionizing Radiation: Levels and Effects. Report to the General Assembly, United Nations, New York, Vol 2, pp 579–655.

U.S. Environmental Protection Agency (1982): Rebuttable presumption against registration of pesticide products containing benomyl. Fed Regist 47:46747.

U.S. Environmental Protection Agency (1984): Proposed guidelines for mutagenicity risk assessment: request for comments. Fed Regist 49(227):46314–46321.

Valencia R, Abrahamson S, Lee W, Von Halle E, Woodruff R, Wurgler F, and Zimmering S (1984): Chromosome mutation tests for mutagenesis in Drosophila melanogaster. Mutat Res 134:61–88.

Vig BK (1984): Sequence of centromere separation: Another mechanism for the origin of nondisjunction. Hum Genet 66:239–243.

Voytek P (1982): Policy and procedures for using mutagenicity data in assessing genetic risk. In Tice RR, Costa DL, Schaich KM (eds): "Genotoxic Effects of Airborne Agents." New York: Plenum, pp 579–588.

Watanabe T, Endo A (1982): Chromosome analysis of preimplantation embryos after cadmium treatment of oocytes at meiosis I. Environ Mutagen 4:563–567.

Waters MD, Auletta A (1981): The Gene-Tox Program: Genetic activity evaluation. J Chem Inf Comput Sci 21:35–38.

Zimmermann FK (1983): Mutagenicity screening with fungal systems. Ann NY Acad Sci 407:186–196.

Zimmermann FK, de Serres FJ, Shelby MD (1979): Workshop on systems to detect induction of aneuploidy by environmental mutagens. Mutat Res 64:279–285.

Zimmering S, Mason JM, Osgood C (1985): Current status of aneuploidy testing in Drosophila (submitted to Mutation Research).

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

This article documents research that was conducted under the auspices of the U.S. Environmental Protection Agency (USEPA). As an arm of the government, the USEPA has a paid up, non-exclusive, irrevocable, worldwide license to reproduce, prepare derivative works, and distribute to the public all of the material contained herein, and to grant others acting on its behalf the right to do so. The USEPA grants to the National Technical Information Service, Department of Commerce, the right to act as its agent in the distribution of this material.